

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

"BA"

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C12N 15/11, 15/12, C07K 14/46, 19/00, 16/18, G01N 33/68, C12Q 1/68	A1	(11) International Publication Number: WO 99/15647 (43) International Publication Date: 1 April 1999 (01.04.99)
(21) International Application Number: PCT/AU98/00795 (22) International Filing Date: 23 September 1998 (23.09.98) (30) Priority Data: PO 9388 23 September 1997 (23.09.97) AU (71) Applicant (for all designated States except US): GARVAN INSTITUTE OF MEDICAL RESEARCH [AU/AU]; c/o St. Vincent's Hospital, 384 Victoria Street, Darlinghurst, NSW 2010 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): DALY, Roger, John [GB/AU]; 49 Gerard Street, Alexandria, NSW 2015 (AU). SUTHERLAND, Robert, Lyndsay [AU/AU]; 20 Northcote Road, Lindfield, NSW 2070 (AU). (74) Agent: F.B. RICE & CO.; 605 Darling Street, Balmain, NSW 2041 (AU).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS (57) Abstract A novel polynucleotide molecule is disclosed which encodes a candidate effector protein for the Grb7 family of signalling proteins. Detection of the protein in a sample such as a homogenised tissue sample should provide a useful tumour marker and/or prognostic indicator for certain human cancers such as breast and prostate cancer.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS

Field of the Invention:

5

The present invention relates to a novel polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins. Detection of the encoded protein in a tissue sample should provide a useful tumour marker and/or prognostic indicator. Furthermore, antagonism of the interaction between Grb7 family members and the encoded protein should provide a novel treatment strategy for human diseases exhibiting aberrant receptor tyrosine kinase (RTK) signalling (e.g. cancer).

15 Background of the Invention

RTKs play a major role in the regulation of cellular growth, differentiation, motility and metabolism by converting an extracellular signal in the form of the binding of a specific hormone or growth factor to the activation of specific signalling pathways and hence modes of intracellular communication (Schlessinger and Ullrich, *Neuron* 9, 383-391, 1992). Activation of RTKs results in both autophosphorylation of the receptor and the phosphorylation of downstream targets on tyrosine residues. It has become evident over the last decade that key elements in receptor-substrate and other protein-protein interactions in RTK signalling are src homology (SH)2 domains. SH2 domains are conserved modules of approximately 100 amino acids found in a wide variety of signalling molecules which bind to short tyrosine-phosphorylated peptide sequences. The specificity of interaction is determined both by the nature of the amino acids flanking the phosphotyrosine residue in the target peptide and residues in the SH2 domain which interact with these sites (Pawson, *Nature* 373, 573-580, 1995).

SH2-domain containing proteins can be divided into two classes: those which possess a catalytic function (e.g. the cytoplasmic tyrosine kinase c-src and the tyrosine phosphatase SH-PTP2) and those which consist entirely of non-catalytic protein domains (eg Grb2), the adaptor sub-class. The function of the latter class is to link separate catalytic subunits to a tyrosine-

35

phosphorylated receptor or signalling intermediate, and other non-catalytic protein modules are often involved in these interactions. For example, SH3 and WW domains (conserved regions of approximately 50 and 40 amino acids, respectively) bind proline-rich peptide ligands, and pleckstrin
5 homology domains (approximately 100 amino acids) interact with both specific phospholipid and protein targets (Pawson, 1995 *supra*).

The Grb7 family represents a family of SH2 domain-containing adaptors which currently contains three members: Grb7, 10 and 14 (Margolis
et al, *Proc. Natl. Acad. Sci. USA* 89, 8894-8898, 1992; Stein *et al*, *EMBO J* 13,
10 1331-1340, 1994; Ooi *et al*, *Oncogene* 10, 1621-1630, 1995; Daly *et al*, *J. Biol. Chem.* 271, 12502-12510, 1996). These proteins share a common overall architecture, consisting of an N-terminal region containing a highly conserved proline-rich decapeptide motif, a central region harbouring a PH domain and a C-terminal SH2 domain. The central region of approximately
15 300 amino acids bears significant homology to the *C. elegans* protein mig10, which is required for long range neuronal migration in embryos, otherwise the Grb7 family and mig10 are structurally distinct. However, they exhibit differences in both SH2 selectivity towards RTKs (Janes *et al*, *J. Biol. Chem.* 272, 8490-8497, 1997) and tissue distribution. The family has therefore
20 evolved to link particular receptors to downstream effectors in a tissue-specific manner. Interestingly, the genes encoding this family appear to have co-segregated with *ERBB* family genes during evolution. Thus *GRB7*, 10 and 14 are linked to *ERBB2*, *ERBB1* (epidermal growth factor receptor) and *ERBB4*, respectively (Stein *et al* 1994 *supra*; Ooi *et al*, 1995 *supra*; Baker *et al*,
25 *Genomics* 36, 218-220, 1996). The juxtaposition of *GRB7* and *ERBB2* leads to common co-amplification in human breast cancers, and since the two gene products are functionally linked, likely up-regulation of an undefined *erbB2* signalling pathway. Furthermore, *GRB14* also exhibits differential expression in human breast cancers (Daly *et al*, 1996 *supra*). These two proteins may
30 therefore modulate RTK signalling in this disease.

In order to identify proteins which bind to this family and therefore identify candidate effectors, we performed a genetic screen using the yeast two hybrid system and Grb14 "bait". This application describes the cloning and characterization of a novel interacting protein, currently designated
35 2.2412.

Disclosure of the Invention:

Thus, in a first aspect, the present invention provides an isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises
5 a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.

Preferably, the polynucleotide molecule comprises a nucleotide sequence having at least 85%, more preferably at least 95%, sequence
10 identity to that shown as SEQ ID NO: 1. Most preferably, the polynucleotide molecule comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

In a preferred embodiment of the invention of the first aspect, the
15 polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.

The polynucleotide molecule may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the activity of endogenous effector proteins of the
20 Grb7 family of signalling proteins.

The polynucleotide molecule may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable host cells such as bacterial, yeast, insect and mammalian host cells. Such host cells may be used to express the protein encoded by the
25 polynucleotide molecule.

Accordingly, in a second aspect, the present invention provides a host cell transformed with the polynucleotide molecule of the first aspect.

In a third aspect, the present invention provides a method of producing a protein, comprising culturing the host cell of the second aspect under
30 conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.

Preferably, the host cell is mammalian or of insect origin. Where the cell is mammalian, it is presently preferred that it be a Chinese hamster ovary (CHO) cell or human embryonic kidney (HEK) 293 cell. Where the
35 host cell is of insect origin, it is presently preferred that it be an insect Sf9 cell.

In a fourth aspect, the present invention provides a purified protein encoded by the polynucleotide molecule of the first aspect.

In a preferred embodiment of this aspect, the purified protein comprises an amino acid sequence substantially corresponding to that shown
5 as SEQ ID NO: 2.

In a fifth aspect, the present invention provides a fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

Fusion proteins according to the fifth aspect may include an N-
10 terminal fragment of a protein such as β -galactosidase to assist in the expression and selection of host cells expressing candidate effector protein, or may include a functional fragment of any other suitable protein to confer additional activity(ies).

In a sixth aspect, the present invention provides an antibody or
15 fragment thereof which specifically binds to the protein of the fourth aspect.

The antibody may be monoclonal or polyclonal, however, it is presently preferred that the antibody is a monoclonal antibody. Suitable antibody fragments include Fab, F(ab')₂ and scFv.

In a seventh aspect, the present invention provides an oligonucleotide
20 probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the polynucleotide molecule of the first aspect under high stringency conditions (Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Second Edition, Cold Spring Harbor
25 Laboratory Press).

In a preferred embodiment of this aspect, the oligonucleotide probe is labelled. In a further preferred embodiment of this aspect, the oligonucleotide probe comprises a nucleotide sequence of at least 18 nucleotides.

In an eighth aspect, the present invention provides a method of
30 detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof of the sixth aspect, and detecting the binding of the antibody or fragment thereof.

The method of the eighth aspect may be conducted using any immunoassays well known in the art (e.g. ELISA). The sample may be, for example, a cell lysate or homogenate prepared from a tissue biopsy.

In a ninth aspect, the present invention provides a method of detecting
5 in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of the seventh aspect, and detecting the binding of the probe.

The method of the ninth aspect may be conducted using any
10 hybridisation assays well known in the art (e.g. Northern blot). The sample may be a poly(A) RNA preparation or homogenate prepared from a tissue biopsy.

Grb7 family proteins exhibit differential expression in certain human cancers (particularly breast and prostate cancer) and may therefore be
15 involved in tumour progression. Detection of the protein encoded by the cDNA 2.2412 in a sample should provide a useful tumour marker and/or prognostic indicator for these cancers. Furthermore, the interaction of Grb7 family members with 2.2412 may provide a novel target for therapeutic intervention.

20 It is to be understood that methods of detecting suitable agonists and methods of therapy utilising detected agonists also form part of the present invention.

The term "substantially corresponds" as used herein in relation to the nucleotide sequence shown as SEQ ID NO: 1 is intended to encompass minor
25 variations in the nucleotide sequence which due to degeneracy in the DNA code do not result in a change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded
30 protein.

The term "substantially corresponding" as used herein in relation to the amino acid sequences shown as SEQ ID NO: 2 is intended to encompass
minor variations in the amino acid sequences which do not result in a decrease in biological activity of the protein. These variations may include
35 conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M: D, E; N, Q; S, T; K, R, H; F, Y, W, H; and

P. N α -alkalamino acids.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature of group of steps, components of features with or without the inclusion of a further step, component or feature or group of steps, components or features.

The invention will hereinafter be described with reference to the accompanying figure and the following, non-limiting example.

Brief description of the accompanying figure:

Figure 1 provides the nucleotide and amino acid (single letter code) sequence of 2.2412. Numbers refer to distances in base pairs. Ankyrin-type repeat sequences are underlined. An additional repeat sequence is indicated by italics. The stop codon is represented by an asterisk. The original cDNA clone 2.2412 isolated by the two hybrid screen spans nucleotides 694-2664 of this sequence.

Figure 2 provides a map of the 2.2412-binding region on Grb14.

A. Structure of the deletion constructs used in the analysis. Gal4 DNA-BD fusion constructs encoding full length Grb14 (FL), the N-terminal (N), central region (C) and N-terminal + central region (N + C) were generated in the vector pAS2.1.

B. Results of β -galactosidase activity assays following transformation of the above plasmids into yeast strain Y190 together with the original 2.2412 cDNA clone in pACT-2.

Example: CLONING AND CHARACTERISATION OF 2.2412

Yeast two hybrid screen

The yeast two hybrid system exploits protein-protein interactions to reconstitute a functional transcriptional activator which can then be detected using a gene reporter system (Fields and Sternglanz, *TIG*, 10, 286-292, 1994). The technique takes advantage of the properties of the Gal4 protein of the yeast *S. cerevisiae*. The Gal4 DNA binding domain (DNA-BD) or activation domain (AD) alone are incapable of inducing transcription. However, an interaction between two proteins synthesized as DNA-BD- and AD-fusions, respectively, brings the Gal4 domains into close proximity and results in

transcriptional activation of two reporter genes (*HIS3* and *LacZ*) which can be monitored by growth on selective medium and biochemical assays.

A plasmid construct encoding a Gal4 DNA-BD-Grb14 fusion was generated as follows. The plasmid *GRB14/pRcCMVF* containing full length
5 *GRB14* cDNA (Daly *et al.* 1996) was restricted with *HindIII* and Klenow treated to create blunt ends, and then digested with *BclI* to release three fragments of approximately 1.1, 4.2 and 1.7 kb. The 1.7 kb fragment was isolated and cloned into the *NdeI* (Klenow treated) and *BamHI* sites of the yeast expression vector pAS2.1 (Clontech) to generate *GRB14/pAS2.1*
10 containing an in-frame fusion of full length Grb14 with the GAL4 DNA-BD. This construct was introduced by electroporation into the yeast strain CG1945 (*MATa*, *ura3-52*, *his3-200*, *ade2-101*, *lys2-801*, *trp1-901*, *leu2-3, 112*, *gal4-542*, *gal80-538*, *cyh1-2*, *LYS2::GAL1UAS-GAL1TATA-HIS3*, *URA3::GAL417mers(x3)-CYC1TATA-lacZ*) selecting for tryptophan
15 prototrophy. The expression of the fusion protein was verified by Western blot analysis with antibodies directed against the Flag epitope and the Gal4 DNA-BD. The recipient strain was then grown to mid-log phase and a human liver cDNA library in the vector pACT2 (Clontech) introduced using the LiAc procedure (Schiestl and Gietz, *Curr. Genet.* 16, 339-346, 1989). Transformants
20 were then selected for tryptophan, leucine and histidine prototrophy in the presence of 5mM 3-aminotriazole.

From a screen of 1×10^6 clones, 39 colonies were initially selected on synthetic complete (SC)-leu-his-trp + 3AT medium and were then tested for β -galactosidase activity. 12 clones scored positive in the latter assay and were
25 subjected to cycloheximide (CHX) curing to remove the bait plasmid by streaking out on SC-leu media containing 10ug/ml CHX (pAS2-1 contains the *CYH2* gene which restores CHX sensitivity to CG1945 cells). This enabled confirmation of the bait dependency of *LacZ* activation and subsequent isolation of the pACT2 plasmids encoding interacting proteins by standard
30 methodology (Philippsen *et al.* *Methods in Enzymology* 194, 170-177). Back transformations were then performed in which these pACT2 plasmids were introduced into CG1945 strains containing the bait plasmid (*GRB14/pAS2-1*) or constructs encoding non-related Gal4 DNA-BD fusions in order to confirm the specificity of the interactions.

35 The DNA sequences of the cDNA inserts were then obtained by cycle sequencing (f-mol kit, Promega) using pACT2-specific and/or clone-specific

primers. Based on their nucleotide sequences the 12 interacting clones were classified into 6 independent groups (see Table I).

TABLE I: Characterization of cDNA clones isolated by the yeast two hybrid screen.

	Class	No. of clones	Identity	Mean RLU (Liquid assay)	Colour intensity (Filter assay)
10	1	6	Nedd4	2.86×10^6	++++
	2	2	Htk	1.86×10^5	++
	3	1	2.2412	5.18×10^6	++++
	4	1	Proteosome	3.88×10^2	+/-
	5	1	Somatostatin	1.45×10^3	+/-
15			receptor		
	6	1	L-arginine:glycine amidinotransferase	8.61×10^2	+/-

The 12 clones exhibiting activation of both the *HIS3* and *lacZ* reporter genes were divided into 6 groups by sequence analysis of their cDNA inserts. Results of β -galactosidase activity assays performed using two methodologies are shown. The liquid culture-derived method (Galacto-Light, TROPIX) is more quantitative; results are given in mean relative light units (RLU) and are normalized for the protein content of the samples. Blue/white screening of the cDNA clones was also performed using a colony lift filter assay (Clontech). The intensity of blue colour development over approximately 2h is scored from +/- (very weak) to + + + (strong).

Six clones were partial cDNAs corresponding to Nedd4, a multidomain protein containing a calcium-dependent phospholipid binding (CaLB) domain, four WW domains and a C-terminal region homologous to the E6-AP carboxyl-terminus (Kumar *et al*, *Biochem. Biophys. Res. Commun.* 185, 1155-1161, 1992; Sudol *et al* *J. Biol. Chem.* 270, 14733-14741, 1995; Huibregtse *et al* *Proc. Natl. Acad. Sci. USA* 92, 2563-2567, 1995). The latter is likely to confer E3 ubiquitin-protein ligase activity on Nedd4. The pACT2 clones isolated encoded the CaLB domain together with the first 22 amino acids of the first WW domain.

Two clones encoded the intracellular region and part of the extracellular domain of Htk, which is a RTK of the Eph family (Bennett *et al* *J. Biol. Chem.* 269, 14211-14218, 1994). The recruitment of Grb14 by Htk is of interest for two reasons. First, the expression profile of both Htk and the murine homologue myk-1 are indicative of a potential role in mammary gland development and neoplasia (Andres *et al* *Oncogene* 9, 1461-1467, 1994; Berclaz *et al* *Biochem. Biophys. Res. Comm.* 226, 869-875, 1996). Second, Eph family members may be involved in the regulation of cell migration (Tessier-Lavigne, *Cell* 82, 345-348, 1995), which is intriguing given the homology of the Grb7 family to the *C. elegans* protein mig10 (Stein *et al.* 1994 *supra*).

A novel cDNA of 1971 bp, designated 2.2412, was also isolated. This clone encoded a polypeptide of 657 amino acids in frame with the Gal4 DNA-BD. The cDNA did not contain a stop codon, and this, together with the Northern analysis described below, indicated that it was incomplete. This DNA fragment was therefore used as a probe to screen a human placental cDNA library (5' STRETCH PLUS, Clontech, in λ gt10). This resulted in the isolation of two clones, designated clone 8 and clone 12. Clone 8 was approximately 2 kb and overlapped the original 2.2412 clone by 900 bp at the 3' end. This clone provided the carboxy-terminal end of the 2.2412 protein sequence (Figure 1). Clone 12 was approximately 3.5 kb and to date has provided an additional 692 bp of sequence information in the 5' direction. The nucleotide and protein sequence for 2.2412 provided by these overlapping clones is shown in Figure 1. Since a 5' initiation codon has yet to be identified the coding sequence still appears to be incomplete.

Further characterization of 2.2412

Database searches using the 2.2412 cDNA sequence revealed significant homology with a large number of proteins containing ankyrin-like repeats. These sequences were first identified as homologous regions between certain cell cycle regulatory proteins and the *Drosophila* protein Notch (Breedon and Nasmyth, *Nature* 329, 651-654, 1987) but subsequently they have been identified in a wide variety of other proteins where they are thought to function in protein-protein interactions (Bork, *Proteins* 17, 363-374, 1993). Subsequent analysis of the protein sequence identified 18 consecutive ankyrin repeats and an additional repetitive element (Figure 1). The ankyrin repeat region is followed by a stretch of approximately 40 amino

acids rich in serine residues. The remaining C-terminal region has a relatively high content of charged amino acids.

Northern analysis of 2.2412 mRNA expression

5 Northern blot analysis of multiple tissue northern (Clontech) was performed using the original 2.2412 cDNA as a probe. This resulted in the detection of a single mRNA transcript of approximately 7 kb in all tissues examined with the exception of the kidney. Expression was particularly high in skeletal muscle and placenta. The size of this transcript compared to that
10 of the 2.2412 clone indicates that the latter represents only a partial cDNA.

Genomic localization of the 2.2412 gene

Fluorescence *in situ* hybridization of the original 2.2412 cDNA to normal metaphases (Baker *et al*, 1996 *supra*) and reference to the FRA10A
15 fragile site at 10q23.32 localized the gene to between chromosome 10q23.2 and proximal 10q23.32. Interestingly, deletions in the 10q22-25 region of chromosome 10 have been detected in a variety of human cancers including breast, prostate, renal, small cell lung and endometrial carcinomas, glioblastoma multiforme, melanoma and meningiomas, suggesting the
20 presence of one or more tumour suppressive loci in this region (Li *et al*, *Science* 275, 1943-1947, 1997; Steck *et al*, *Nature Genetics* 15, 356-362, 1997, and references therein). Two candidate tumour suppressor genes have been identified in this region (MMAC1/PTEN and MXI1. Li *et al* 1997 *supra*; Steck *et al* 1997 *supra*; Albarosa *et al*, *Hum. Genet.* 95, 709-711, 1995).

25

Analysis of the interaction between 2.2412 and Grb7 family members

cDNAs encoding the full length and N- and C-terminal regions of the original 2.2412 cDNA clone (nucleotides 694-2664, 694-1614 and 1615-2664 of the sequence shown in Figure 1, respectively) were cloned into the vector
30 pGEX4T2 (Pharmacia). The full length construct was generated by subcloning from the pACT2 clone as a NdeI fragment, whereas the shorter constructs were synthesized by directional cloning of PCR products. The corresponding GST-fusion proteins were purified from IPTG-induced bacterial cultures using glutathione-agarose beads (Smith and Johnson, *Gene*
35 67, 31-40, 1988). These immobilized fusion proteins were then incubated with lysates from cells expressing Flag epitope-tagged Grb14 (Daly *et al*, 1996

supra) or human breast cancer cells expressing high levels of Grb7 (SK-BR-3; Stein *et al.* 1994) as described previously (Daly *et al.* 1996). Following washing, bound proteins were detected by Western blot analysis. The results indicated that 2.2412 bound specifically to both Grb14 and Grb7 *in vitro*, and
5 that the N-terminal fusion protein bound more strongly than that derived from the C-terminus. These data, obtained using a different methodology for detecting protein-protein interactions to the yeast two hybrid system, confirm that 2.2412 interacts with Grb14. Furthermore, 2.2412 also binds Grb7. Consequently 2.2412 appears to represent a general effector for the Grb7
10 family.

Mapping of the 2.2412 binding region on Grb14

In order to identify the region of Grb14 that interacts with 2.2412, a series of Grb14 deletion mutants were generated by cloning PCR fragments
15 synthesized using the appropriate flanking primers into the vector pAS2.1. These fragments spanned the following regions: N-terminus ("N", amino acids 1-110), the central region ("C") encompassing the mig10 homology and the "between PH and SH2" (BPS) domain (amino acids 110-437) and the N-terminal and central regions ("N + C", amino acids 1-437). These plasmids
20 were individually transformed into the yeast strain Y190 (*MATa*, *ura3-52*, *his3-200*, *ade2-101*, *lys2-801*, *trp1-901*, *leu2-3, 112*, *gal4Δ*, *gal80Δ*, *cyh^r2*, *LYS2::GAL1UAS-HIS3TATA-HIS3*, *URA3::GAL1UAS-GAL1TATA-lacZ*) and expression of the appropriately sized Gal4 DNA-BD fusion proteins confirmed by Western blotting. Following transformation of the resulting
25 yeast strains with the original 2.2412 cDNA clone in pACT-2, the strength of the interaction was determined by either liquid- or filter-based β -galactosidase assays. The results are presented in Figure 2, and demonstrate that the N-terminal region of Grb14 is not only required, but is also sufficient, for binding 2.2412. This supports the hypothesis that 2.2412
30 represents a general effector for the Grb7 family, since the N-terminal region of these proteins contains a highly conserved proline-rich motif which may mediate this interaction.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
5 be considered in all respects as illustrative and not restrictive.

Sequence listings:**SEQUENCE LISTING**

Applicant: Garvan Institute of Medical Research

Title of Invention: A potential effector for the Grb7 family of signalling proteins.

Current Application Number:

Current Filing Date:

Prior Application Number: PO9388

Prior Application Filing Date: 1997-09-23

Number of ID SEQ Nos: 2

Software: PatentIn Ver. 2.0

SEQ ID NO: 1

Length: 3400

Type: DNA

Organism: Homo sapiens

Sequence: 1

```

attcctcttc ataatgcatg ctcttttggg catgctgaag tagtcaatct cctttttgcga 60
catggtgcag accccaatgc tcgagataat tggaattata ctctctcca tgaagctgca 120
attaaaggaa agattgatgt ttgcattgtg ctgttacagc atggagctga gcccaaccatc 180
cgaaatacag atggaaggac agcattggat ttagcagatc catctgccaa agcagtgctt 240
actggtgaat ataagaaaga tgaactctta gaaagtgccg ggagtggcaa tgaagaaaaa 300
atgatggctc tactcacacc attaaatgtc aactgccacg caagtgatgg cagaaagtca 360
actccattac atttggcagc aggatataac agagtaaaga ttgtacagct gttactgcaa 420
catggacgtg atgtccatgc taaagataaa ggtgatctgg taccattaca caatgcctgt 480
tcttatggtc attatgaagt aactgaactt ttggtcaagc atggtggctg tgtaaatgca 540
atggacttgt ggcaattcac tctcttcat gaggcagctt ctaagaacag ggttgaagta 600
tggtctcttc tettaagtta tggtgcagac ccaacactgc tcaattgtaa gaataaaagt 660
gctatagact tggtccccc accacagtta aaagaaagat tagcatatga atttaaaggc 720
cactcgttgc tgcaagctgc acgagaagct gatgttactc gaatcaaaaa acatctctct 780
ctggaatagg tgaatttcaa gcctcctcaa acacatgaaa cagcattgca ttgtgctgct 840
gcctctccat atcccaaaa aaagcaataa tgtgaactgt tgctaagaaa aggagcaaac 900
atcaatgaaa agactaaaga attcttgact cctctgcacg tggcatctga gaaagctcat 960
aatgatgttg ttgaagtagt ggtgaaacat gaagcaaagg ttaatgctct ggataatctt 1020
ggtcagactt ctctacacag agctgcatat tgtggtcctc taaaaacctg ccgcctactc 1080
ctgagctatg ggtgtgatcc taacattata tcccttcagg gctttactgc ttacagatg 1140
ggaaatgaaa atgtacagca actcctccaa gagggatatc cattaggtaa ttcagaggca 1200
gacagacaat tgctggaagc tgcaaaggct ggagatgtcg aaactgtaaa aaaactgtgt 1260
actgttcaga gtgtcaactg cagagacatt gaagggcgct agtctacacc acttcatttt 1320
gcagctgggt ataacagagt gtccgtggtg gaatatctgc tacagcatgg agctgatgtg 1380
catgctaaa agataaaggagg ccttgtaact ttgcacaatg catgttctta cggacattat 1440
gaagttgcag aactcttgtt taaacatgga gcagtagtta atgtagctga tttatggaaa 1500
tttacacctt tacatgaagc agcagcaaaa ggaaaatatg aaatttgcaa acttctgctc 1560
cagcatgggt cagaccctac aaaaaaaaaa agggatggaa ataactctt ggatcttgtt 1620
aaagatggag atacagatat tcaagatctg cttaggggag atgcagcttt gctagatgct 1680
gccaagaagg gttgttttagc cagagtgaag aagttgtctt ctctgataa tgtaaatgct 1740
cgcatatccc aaggcagaca ttcaacacct ttacatttag cagctgggta taataattta 1800
gaagttgcag agtatttgtt acaacacgga gctgatgtga atgcccaga caaaggagga 1860
cttattcctt tacataatgc aqcatcttac gggcatgtag atgtagcagc tctactaata 1920

```

```

aagtataatg catctctcaa tgcacaggac aaatggggtt tccacacctt gcacgaagca 1980
gccccaaaagg gacgaacaca gctttgtgct ttgttgctag cccatggagc tgacccgact 2040
cttaaaaaatc aggaaggaca aacaccttta gatttagttt cagcagatga tgtcagcgct 2100
cttctgacag cagccatgcc cccatctgct ctgcccctctt gttacaagcc tcaagtgtct 2160
aatgggtgtga gaagcccagg agccactgca gatgctctct cttcaggtcc atctagccca 2220
tcaagcccttt ctgcagccag cagtcttgac aacttatctg ggagtttttc agaactgtct 2280
tcagtagtta gttcaagtgg aacagagggt gcttccagtt tggagaaaaa ggaggttcca 2340
ggagtagatt ttagcataac tcaattcgta aggaatcttg gacttgagca cctaattgat 2400
atatttgaga gagaacagat cactttggat gtattagttg agatggggca caaggagctg 2460
aaggagattg gaatcaatgc ttatggacat aggcacaaac taattaaagg agtcgagaga 2520
cttatctccg gacaacaagg tcttaaccca tatttaactt tgaacacctc tggtagtggg 2580
acaattctta tagatctgtc tctgatgat aaagagtttc agtctgtgga ggaagagatg 2640
caaagtacag ttcgagagca cagagatgga ggtcatgcag gtggaatctt caacagatac 2700
aatattctca agattcagaa ggtttgtaac aagaaactat gggaaagata cactcaccgg 2760
agaaaagaag tttctgaaga aaaccacaac catgccaatg aacgaatgct atttcatggg 2820
tctccttttg tgaatgcaat tatccacaaa ggctttgatg aaaggcatgc gtacataggt 2880
ggtatgtttg gagctggcat ttattttgct gaaaactctt ccaaaaagca tcaatatgta 2940
tatggaattg gaggaggtac tgggtgtcca gttcacaaaag acagatcttg ttacatttgc 3000
cacaggcagc tgcctctttg cgggtaacc ttgggaaagt ctttctgca gttcagtga 3060
atgaaaatgg cacattctcc tccaggtcat cactcagtea ctggtaggcc cagtgtaaat 3120
ggcctagcat tagctgaata tgttatttac agaggagaac aggcctatcc tgagtattta 3180
attacttacc agattatgag gcttgaaggt atggtcgatg gataaatagt tattttaaga 3240
aactaattcc actgaacctt aaatcatcaa agcagcagtg gcctctacgt tttactcctt 3300
tgctgaaaaa aaatcatctt gccacaggc ctgtggcaaa aggataaaaa tgtgaacgaa 3360
gtttaacatt ctgacttgat aaagctttaa taatgtacag 3400

```

SEQ ID NO: 2

Length: 1074

Type: PRT

Organism: Homo sapiens

Sequence: 2

Ile Pro Leu His Asn Ala Cys Ser Phe Gly His Ala Glu Val Val Asn
 1 5 10 15

Leu Leu Leu Arg His Gly Ala Asp Pro Asn Ala Arg Asp Asn Trp Asn
 20 25 30

Tyr Thr Pro Leu His Glu Ala Ala Ile Lys Gly Lys Ile Asp Val Cys
 35 40 45

Ile Val Leu Leu Gln His Gly Ala Glu Pro Thr Ile Arg Asn Thr Asp
 50 55 60

Gly Arg Thr Ala Leu Asp Leu Ala Asp Pro Ser Ala Lys Ala Val Leu
 65 70 75 80

Thr Gly Glu Tyr Lys Lys Asp Glu Leu Leu Glu Ser Ala Arg Ser Gly
 85 90 95

Asn Glu Glu Lys Met Met Ala Leu Leu Thr Pro Leu Asn Val Asn Cys
 100 105 110

His Ala Ser Asp Gly Arg Lys Ser Thr Pro Leu His Leu Ala Ala Gly
 115 120 125

Tyr Asn Arg Val Lys Ile Val Gln Leu Leu Leu Gln His Gly Arg Asp
 130 135 140

Val His Ala Lys Asp Lys Gly Asp Leu Val Pro Leu His Asn Ala Cys

145 150 155 160
 Ser Tyr Gly His Tyr Glu Val Thr Glu Leu Leu Val Lys His Gly Gly
 165 170 175
 Cys Val Asn Ala Met Asp Leu Trp Gln Phe Thr Pro Leu His Glu Ala
 180 185 190
 Ala Ser Lys Asn Arg Val Glu Val Cys Ser Leu Leu Leu Ser Tyr Gly
 195 200 205
 Ala Asp Pro Thr Leu Leu Asn Cys Lys Asn Lys Ser Ala Ile Asp Leu
 210 215 220
 Ala Pro Thr Pro Gln Leu Lys Glu Arg Leu Ala Tyr Glu Phe Lys Gly
 225 230 235 240
 His Ser Leu Leu Gln Ala Ala Arg Glu Ala Asp Val Thr Arg Ile Lys
 245 250 255
 Lys His Leu Ser Leu Glu Met Val Asn Phe Lys His Pro Gln Thr His
 260 265 270
 Glu Thr Ala Leu His Cys Ala Ala Ala Ser Pro Tyr Pro Lys Arg Lys
 275 280 285
 Gln Ile Cys Glu Leu Leu Leu Arg Lys Gly Ala Asn Ile Asn Glu Lys
 290 295 300
 Thr Lys Glu Phe Leu Thr Pro Leu His Val Ala Ser Glu Lys Ala His
 305 310 315 320
 Asn Asp Val Val Glu Val Val Val Lys His Glu Ala Lys Val Asn Ala
 325 330 335
 Leu Asp Asn Leu Gly Gln Thr Ser Leu His Arg Ala Ala Tyr Cys Gly
 340 345 350
 His Leu Gln Thr Cys Arg Leu Leu Leu Ser Tyr Gly Cys Asp Pro Asn
 355 360 365
 Ile Ile Ser Leu Gln Gly Phe Thr Ala Leu Gln Met Gly Asn Glu Asn
 370 375 380
 Val Gln Gln Leu Leu Gln Glu Gly Ile Ser Leu Gly Asn Ser Glu Ala
 385 390 395 400
 Asp Arg Gln Leu Leu Glu Ala Ala Lys Ala Gly Asp Val Glu Thr Val
 405 410 415
 Lys Lys Leu Cys Thr Val Gln Ser Val Asn Cys Arg Asp Ile Glu Gly
 420 425 430
 Arg Gln Ser Thr Pro Leu His Phe Ala Ala Gly Tyr Asn Arg Val Ser
 435 440 445
 Val Val Glu Tyr Leu Leu Gln His Gly Ala Asp Val His Ala Lys Asp
 450 455 460
 Lys Gly Gly Leu Val Pro Leu His Asn Ala Cys Ser Tyr Gly His Tyr

465 470 475 480
 Glu Val Ala Glu Leu Leu Val Lys His Gly Ala Val Val Asn Val Ala
 485 490 495
 Asp Leu Trp Lys Phe Thr Pro Leu His Glu Ala Ala Ala Lys Gly Lys
 500 505 510
 Tyr Glu Ile Cys Lys Leu Leu Leu Gln His Gly Ala Asp Pro Thr Lys
 515 520 525
 Lys Asn Arg Asp Gly Asn Thr Pro Leu Asp Leu Val Lys Asp Gly Asp
 530 535 540
 Thr Asp Ile Gln Asp Leu Leu Arg Gly Asp Ala Ala Leu Leu Asp Ala
 545 550 555 560
 Ala Lys Lys Gly Cys Leu Ala Arg Val Lys Lys Leu Ser Ser Pro Asp
 565 570 575
 Asn Val Asn Cys Arg Asp Thr Gln Gly Arg His Ser Thr Pro Leu His
 580 585 590
 Leu Ala Ala Gly Tyr Asn Asn Leu Glu Val Ala Glu Tyr Leu Leu Gln
 595 600 605
 His Gly Ala Asp Val Asn Ala Gln Asp Lys Gly Gly Leu Ile Pro Leu
 610 615 620
 His Asn Ala Ala Ser Tyr Gly His Val Asp Val Ala Ala Leu Leu Ile
 625 630 635 640
 Lys Tyr Asn Ala Ser Leu Asn Ala Thr Asp Lys Trp Ala Phe Thr Pro
 645 650 655
 Leu His Glu Ala Ala Gln Lys Gly Arg Thr Gln Leu Cys Ala Leu Leu
 660 665 670
 Leu Ala His Gly Ala Asp Pro Thr Leu Lys Asn Gln Glu Gly Gln Thr
 675 680 685
 Pro Leu Asp Leu Val Ser Ala Asp Asp Val Ser Ala Leu Leu Thr Ala
 690 695 700
 Ala Met Pro Pro Ser Ala Leu Pro Ser Cys Tyr Lys Pro Gln Val Leu
 705 710 715 720
 Asn Gly Val Arg Ser Pro Gly Ala Thr Ala Asp Ala Leu Ser Ser Gly
 725 730 735
 Pro Ser Ser Pro Ser Ser Leu Ser Ala Ala Ser Ser Leu Asp Asn Leu
 740 745 750
 Ser Gly Ser Phe Ser Glu Leu Ser Ser Val Val Ser Ser Ser Gly Thr
 755 760 765
 Glu Gly Ala Ser Ser Leu Glu Lys Lys Glu Val Pro Gly Val Asp Phe
 770 775 780
 Ser Ile Thr Gln Phe Val Arg Asn Leu Gly Leu Glu His Leu Met Asp

785 790 795 800
 Ile Phe Glu Arg Glu Gln Ile Thr Leu Asp Val Leu Val Glu Met Gly
 805 810 815
 His Lys Glu Leu Lys Glu Ile Gly Ile Asn Ala Tyr Gly His Arg His
 820 825 830
 Lys Leu Ile Lys Gly Val Glu Arg Leu Ile Ser Gly Gln Gln Gly Leu
 835 840 845
 Asn Pro Tyr Leu Thr Leu Asn Thr Ser Gly Ser Gly Thr Ile Leu Ile
 850 855 860
 Asp Leu Ser Pro Asp Asp Lys Glu Phe Gln Ser Val Glu Glu Glu Met
 865 870 875 880
 Gln Ser Thr Val Arg Glu His Arg Asp Gly Gly His Ala Gly Gly Ile
 885 890 895
 Phe Asn Arg Tyr Asn Ile Leu Lys Ile Gln Lys Val Cys Asn Lys Lys
 900 905 910
 Leu Trp Glu Arg Tyr Thr His Arg Arg Lys Glu Val Ser Glu Glu Asn
 915 920 925
 His Asn His Ala Asn Glu Arg Met Leu Phe His Gly Ser Pro Phe Val
 930 935 940
 Asn Ala Ile Ile His Lys Gly Phe Asp Glu Arg His Ala Tyr Ile Gly
 945 950 955 960
 Gly Met Phe Gly Ala Gly Ile Tyr Phe Ala Glu Asn Ser Ser Lys Ser
 965 970 975
 Asn Gln Tyr Val Tyr Gly Ile Gly Gly Gly Thr Gly Cys Pro Val His
 980 985 990
 Lys Asp Arg Ser Cys Tyr Ile Cys His Arg Gln Leu Leu Phe Cys Arg
 995 1000 1005
 Val Thr Leu Gly Lys Ser Phe Leu Gln Phe Ser Ala Met Lys Met Ala
 1010 1015 1020
 His Ser Pro Pro Gly His His Ser Val Thr Gly Arg Pro Ser Val Asn
 1025 1030 1035 1040
 Gly Leu Ala Leu Ala Glu Tyr Val Ile Tyr Arg Gly Glu Gln Ala Tyr
 1045 1050 1055
 Pro Glu Tyr Leu Ile Thr Tyr Gln Ile Met Arg Pro Glu Gly Met Val
 1060 1065 1070
 Asp Gly

Claims:

1. An isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.
2. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 85% sequence identity to that shown as SEQ ID NO: 1.
3. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 95% sequence identity to that shown as SEQ ID NO: 1.
4. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.
5. A host cell transformed with a polynucleotide molecule according to any one of the preceding claims.
6. A host cell according to claim 5, wherein the host cell is a mammalian, insect, yeast or bacterial host cell.
7. A method of producing a protein, comprising culturing the host cell of claim 5 or 6 under conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.
8. A purified protein encoded by a polynucleotide molecule according to any one of claims 1 to 4.
9. A purified protein according to claim 8, wherein the protein comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

10. A fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.
11. An antibody or fragment thereof which specifically binds to a protein
5 according to claim 8 or 9.
12. An oligonucleotide probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the
10 polynucleotide molecule of any one of claims 1 to 4 under high stringency conditions.
13. An oligonucleotide probe according to claim 12. wherein the oligonucleotide probe comprises a nucleotide sequence of at least 18
15 nucleotides.
14. A method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof according to claim 11.
20
15. A method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of claim 12 or 13.

1/4

FIGURE 1

ATTCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCCTTTTGGACATGGTGCAG 70
I P L H N A C S F G H A E V V N L L L R H G A

ACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATGAAGCTGCAATTAAGGAAAGATTGATGT 140
D P N A R D N W N Y T P L H E A A I K G K I D V

TTGCATTGTGCTGTTACAGCATGGAGCTGAGCCAACCATCCGAAATACAGATGGAAGGACAGCATTGGAT 210
C I V L L Q H G A E P T I R N T D G R T A L D

TTAGCAGATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGTGCCA 280
L A D P S A K A V L T G E Y K K D E L L E S A

GGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAAATGTCAACTGCCACGCAAGTGATGG 350
R S G N E E K M M A L L T P L N V N C H A S D G

CAGAAAGTCAACTCCATTACATTTGGCAGCAGGATATAACAGAGTAAAGATTGTACAGCTGTTACTGCAA 420
R K S T P L H L A A G Y N R V K I V Q L L L Q

CATGGACGTGATGTCCATGCTAAAGATAAAGTGATCTGGTACCATTACACAATGCCTGTTCTTATGGTC 490
H G R D V H A K D K G D L V P L H N A C S Y G

ATTATGAAGTAACTGAACTTTTGGTCAAGCATGGTGGCTGTGTAATGCAATGGACTTGTGGCAATTAC 560
H Y E V T E L L V K H G G C V N A M D L W Q F T

TCCTCTTCATGAGGCAGCTTCTAAGAACAGGGTTGAAGTATGTTCTCTTCTCTTAAGTTATGGTGCAGAC 630
P L H E A A S K N R V E V C S L L L S Y G A D

CCAACACTGCTCAATTGTAAGAATAAAAGTGCTATAGACTTGGCTCCACACCACAGTTAAAGAAAGAT 700
P T L L N C K N K S A I D L A P T P Q L K E R

TAGCATATGAATTTAAAGGCCACTCGTTGCTGCAAGCTGCACGAGAAGCTGATGTTACTCGAATCAAAAA 770
L A Y E F K G H S L L Q A A R E A D V T R I K K

ACATCTCTCTCTGGAATGGTGAATTTCAAGCATCCTCAAAACACATGAAACAGCATTGCATTGTGCTGCT 840
H L S L E M V N F K H P Q T H E T A L H C A A

GCATCTCCATATCCCAAAAGAAAGCAAATATGTGAAGTGTGCTAAGAAAAGGAGCAAACATCAATGAAA 910
A S P Y P K R K Q I C E L L L R K G A N I N E

AGACTAAAGAATTCTTGACTCCTCTGCACGTGGCATCTGAGAAAGCTCATAATGATGTTGTTGAAGTAGT 980
K T K E F L T P L H V A S E K A H N D V V E V V

GGTGAACATGAAGCAAAGGTTAATGCTCTGGATAATCTTGGTCAGACTTCTCTACACAGAGCTGCATAT 1050
V K H E A K V N A L D N L G Q T S L H R A A Y

TGTGGTCATCTACAAACCTGCCGCTACTCCTGAGCTATGGGTGTGATCCTAACATTATATCCCTTCAGG 1120
C G H L Q T C R L L L S Y G C D P N I I S L Q

GCTTTACTGCTTTACAGATGGGAAATGAAATGTACAGCAACTCCTCCAAGAGGGTATCTCATTAGGTAA 1190
G F T A L Q M G N E N V Q Q L L Q E G I S L G N

TTCAGAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGATGTCGAAACTGTAAAAAACTGTGT 1260
S E A D R Q L L E A A K A G D V E T V K K L C

ACTGTTTCAGAGTGTCAACTGCAGAGACATTGAAGGGCGTCAGTCTACACCATTCTTTTGCAGCTGGGT 1330
T V Q S V N C R D I E G R Q S T P L H F A A G

ATAACAGAGTGTCCGTGGTGAATATCTGCTACAGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGG 1400
Y N R V S V V E Y L L Q H G A D V H A K D K G G

CCTGTACCTTTGCACAATGCATGTTCTTACGGACATTATGAAGTTGCAGAACTTCTTGTAAACATGGA 1470
L V P L H N A C S Y G H Y E V A E L L V K H G

GCAGTAGTTAATGTAGCTGATTTATGGAAATTTACACCTTACATGAAGCAGCAGCAAAAGGAAAATATG 1540
A V V N V A D L W K F T P L H E A A A K G K Y

AAATTGCAAACTTCTGCTCCAGCATGGTGCAGACCTTACAAAAAAGGAGGATGGAATACTCCTTT 1610
E I C K L L L Q H G A D P T K K N R D G N T P L

2/4

GGATCTTGTTAAAGATGGAGATACAGATATTCAAGATCTGCTTAGGGGAGATGCAGCTTTGCTAGATGCT 1680
D L V K D G D T D I Q D L L R G D A A L L D A

GCCAAGAAGGGTTGTTTAGCCAGAGTGAAGAAGTTGTCTTCTCTGATAATGTAAATTGCCGCGATACCC 1750
A K K G C L A R V K K L S S P D N V N C R D T

AAGGCAGACATTCAACACCTTTACATTTAGCAGCTGGTTATAATAATTTAGAAGTTGCAGAGTATTTGTT 1820
Q G R H S T P L H L A A G Y N N L E V A E Y L L

ACAAACACGGAGCTGATGTGAATGCCCAAGACAAAGGAGGACTTATTCCTTTACATAATGCAGCATCTTAC 1890
Q H G A D V N A Q D K G G L I P L H N A A S Y

GGGCATGTAGATGTAGCAGCTCTACTAATAAAGTATAATGCATCTCTCAATGCCACGGACAAATGGGCTT 1960
G H V D V A A L L I K Y N A S L N A T D K W A

TCACACCTTTGCACGAAGCAGCCCAAGGGACGAACACAGCTTTGTGCTTTGTTGCTAGCCCATGGAGC 2030
F T P L H E A A Q K G R T Q L C A L L L A H G A

TGACCCGACTCTTAAAAATCAGGAAGGACAAACACCTTTAGATTTAGTTTCAGCAGATGATGTCAGCGCT 2100
D P T L K N Q E G Q T P L D L V S A D D V S A

CTTCTGACAGCAGCCATGCCCCATCTGCTCTGCCCTCTTGTTACAGCCTCAAGTCTCAATGGTGTGA 2170
L L T A A M P P S A L P S C Y K P Q V L N G V

GAAGCCAGGAGCCACTGCAGATGCTCTCTCTTCAGGTCCATCTAGCCCATCAAGCCTTTCTGCAGCCAG 2240
R S P G A T A D A L S S G P S S P S S L S A A S

CAGTCTTGACAACTTATCTGGGAGTTTTCAGAACTGTCTTCAGTAGTTAGTTCAAGTGGAAACAGAGGGT 2310
S L D N L S G S F S E L S S V V S S S G T E G

GCTTCCAGTTTGGAGAAAAAGGAGGTTCCAGGAGTAGATTTTAGCATAACTCAATTCGTAAGGAATCTTG 2380
A S S L E K K E V P G V D F S I T Q F V R N L

GACTTGAGCACCTAATGGATATATTTGAGAGAGAACAGATCACTTTGGATGTATTAGTTGAGATGGGGCA 2450
G L E H L M D I F E R E Q I T L D V L V E M G H

CAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGGACATAGGCACAACTAATTAAAGGAGTCGAGAGA 2520
K E L K E I G I N A Y G H R H K L I K G V E R

CTTATCTCCGGACAAACAGGTCTTAAACCATATTTAACTTTGAACACCTCTGGTAGTGAACAATCTTAA 2590
L I S G Q Q G L N P Y L T L N T S G S G T I L

TAGATCTGTCTCCTGATGATAAAGAGTTTCAGTCTGTGGAGGAAGAGATGCAAGTACAGTTTCGAGAGCA 2660
I D L S P D D K E F Q S V E E E M Q S T V R E H

CAGAGATGGAGGTATGCAGGTGGAATCTTCAACAGATACAATATTCTCAAGATTGAGAAGGTTTGTAAAC 2730
R D G G H A G G I F N R Y N I L K I Q K V C N

AAGAACTATGGGAAAGATACACTCACCGGAGAAAAGAAGTTTCTGAAGAAAACCACAACCATGCCAATG 2800
K K L W E R Y T H R R K E V S E E N H N H A N

AACGAATGCTATTTTCATGGGTCTCCTTTTGTGAATGCAATTATCCACAAAGGCTTTGATGAAAGGCATGC 2870
E R M L F H G S P F V N A I I H K G F D E R H A

GTACATAGGTGGTATGTTTGGAGCTGGCATTATTTTGTGAAAACCTCTTCCAAAAGCAATCAATATGTA 2940
Y I G G M F G A G I Y F A E N S S K S N Q Y V

TATGGAATTGGAGGAGGTACTGGGTGTCCAGTTCACAAAGACAGATCTTGTTACATTTGCCACAGGCAGC 3010
Y G I G G G T G C P V H K D R S C Y I C H R Q

TGCTCTTTTGCCGGGTAACTTGGGAAAGTCTTTCCTGCAGTTCAGTGCATGAAAATGGCACATTCTCC 3080
L L F C R V T L G K S F L Q F S A M K M A H S P

TCCAGGTCATCACTCAGTCACTGGTAGGCCAGTGTAATGCCCTAGCATTAGCTGAATATGTTATTTAC 3150
P G H H S V T G R P S V N G L A L A E Y V I Y

AGAGGAGAACAGGCTTATCTGAGTATTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTCGATG 3220
R G E Q A Y P E Y L I T Y Q I M R P E G M V D

GATAAATAGTTATTTTAAGAACTAATTCCTGAACTAAAATCATCAAAGCAGCAGTGGCCTCTACGT 3290
G *

3/4

TTTACTCCTTTGCTGAAAAAAAAATCATCTTGCCACAGGCCTGTGGCAAAGGATAAAAAATGTGAACGAA 3360

GTTTAACATTCTGACTTGATAAAGCTTTAATAATGTACAG

A

CONSTRUCT

STRUCTURE

N



C



N + C



FL

**B**

CONSTRUCT

MEAN RLU
(LIQUID ASSAY)
(X 10³)

COLOUR INTENSITY (FILTER ASSAY)

pAS2.1

4

—

N

109

++

C

3

5

N + C

194

++

FL


242

+++

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 98/00795

A. CLASSIFICATION OF SUBJECT MATTER												
Int Cl ⁶ : C12N 15/11, 15/12; C07K 14/46, 19/00, 16/18; G01N 33/68; C12Q 1/68												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) See Electronic Databases												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Electronic Databases												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) <u>WPAT (DGENE)</u> - SEQ.ID.NO:2; <u>Genbank</u> , <u>EMBL</u> , <u>Swiss-prot</u> , <u>PIR</u> - SEQ.ID.NO:1, SEQ.ID.NO:2; <u>MEDLINE</u> - Grb7, Grb#, growth factor receptor bound												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
A	Janes PW et al. (1997) "Structural determinants of the interaction between the erbB2 receptor and the Src homology 2 domain of Grb7". The Journal of Biological Chemistry volume 272(13) pages 8490-8497. See entire document	1-15										
A	Keegen K and Cooper JA "Use of the two hybrid systems to detect the association of the protein-tyrosine-phosphatase, SHPTP2, with another SH2-containing protein, Grb7" Oncogene volume 12, pages 1537-1544. See entire document	1-15										
<input type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 4 November 1998		Date of mailing of the international search report 11 NOV 1998										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer  JULIE CAIRNDUFF Telephone No.: (02) 6283 2545										